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Cannabinoids for Cancer Treatment: Progress and Promise

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Abstract

Cannabinoids are a class of pharmacologic compounds that offer potential applications as antitumor drugs, based on the ability of some members of this class to limit inflammation, cell proliferation, and cell survival. In particular, emerging evidence suggests that agonists of cannabinoid receptors expressed by tumor cells may offer a novel strategy to treat cancer. Here, we review recent work that raises interest in the development and exploration of potent, nontoxic, and nonhabit forming cannabinoids for cancer therapy. [Cancer Res 2008;68(2):339–42]

Cannabinoid Receptors: A Brief Overview

Cannabinoid refers to a group of chemicals naturally found in the marijuana plant *Cannabis sativa L.* and includes compounds that are either structurally or pharmacologically similar to $\Delta(9)$ -tetrahydrocannabinol or those that bind to the cannabinoid receptors. It was earlier thought that cannabinoids exert their physiologic and behavioral effects via nonspecific interaction with cell membranes. Although anticancer effects of cannabinoids were shown as early as 1975 in Lewis lung carcinoma (ref. 1 and references therein), renewed interest was generated little after the discovery of the cannabinoid system and cloning of the specific cannabinoid receptors (1). The diversified effects of cannabinoids are now known to be mediated through the activation of G-protein-coupled receptors that are normally bound by a family of endogenous ligands, the endocannabinoids (1). Two cannabinoid receptors have been characterized and cloned from mammalian tissues: the “central” CB₁ receptor and the “peripheral” CB₂ receptor. CB₁ receptors are found primarily in the brain, specifically in the basal ganglia and in the limbic system, including the hippocampus. They are also found in the cerebellum and in both male and female reproductive systems. CB₂ receptors are almost exclusively found in the immune system, with the greatest density in the spleen (1).

Classification of Cannabinoids

There are three types of cannabinoids. Plant-derived cannabinoids such as $\Delta(9)$ -tetrahydrocannabinol and cannabidiol occur uniquely in the cannabis plant; endogenous cannabinoids also known as endocannabinoids such as anandamide and 2-arachidonoylglycerol are produced in the bodies of humans and animals; and synthetic cannabinoids, such as WIN-55, 212-2, JWH-133, and (*R*)-methanandamide (MET), which are developed in a laboratory, bear structural similarities to either natural or the endogenous cannabinoids.

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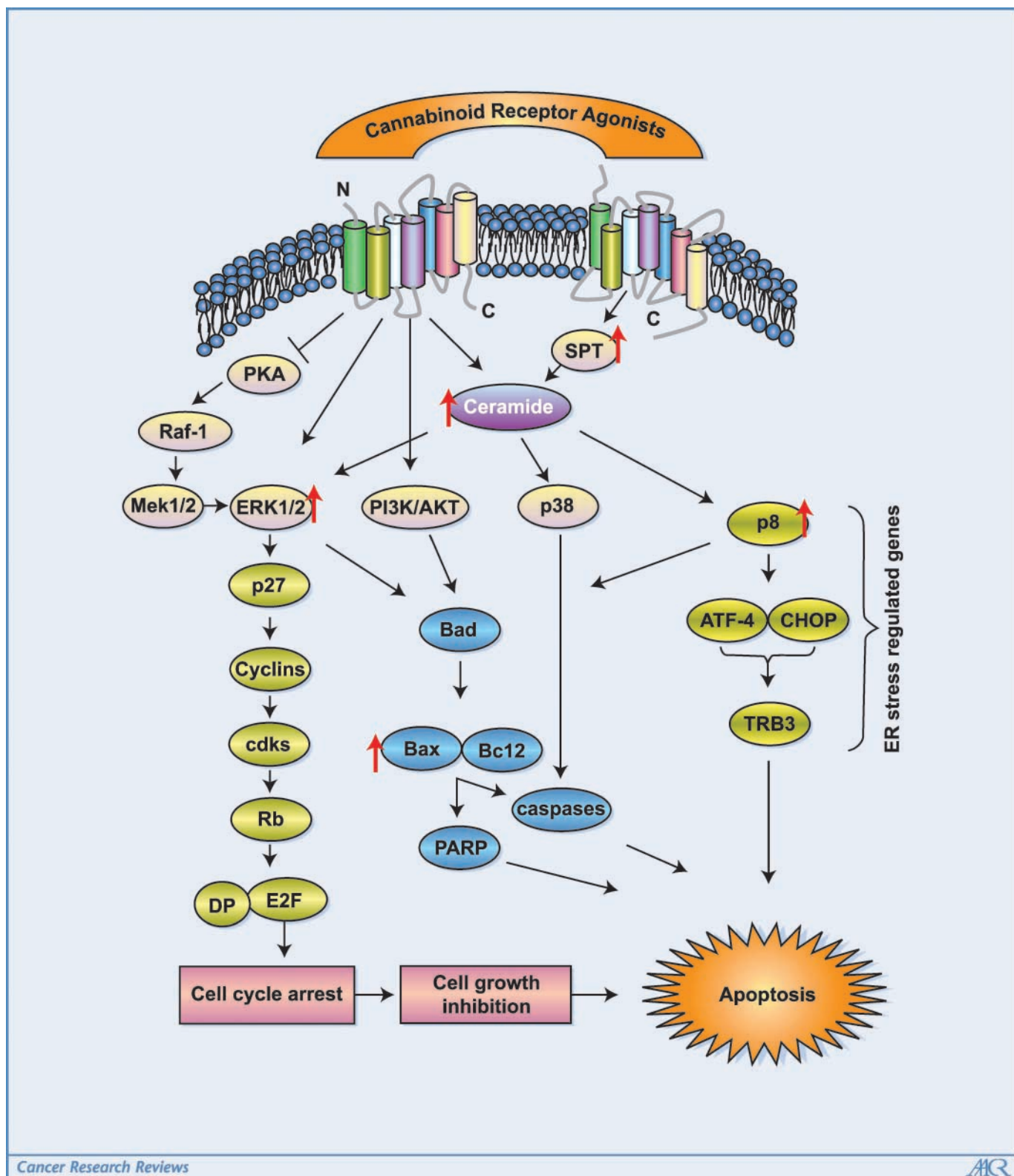
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Cannabinoids in the Treatment of Cancer: Progress

Cancer is a disease characterized by uncontrolled division of cells and their ability to spread. This unregulated growth is caused by damage to DNA, resulting in mutations, defects in cell cycle, and apoptotic machinery. Thus, agents that can modulate apoptosis to maintain steady-state cell population by affecting one or more signaling intermediates leading to induction of apoptosis can be useful for targeted therapy of cancer. Hence, there is a need to develop novel targets and mechanism-based agents for the management of cancer. A significant advancement in cannabinoid use in cancer treatment came through the discovery of a potential utility of these compounds for targeting and killing tumors. In the early 1970s, cannabinoids were shown to inhibit tumor growth and prolong the life of mice bearing Lewis lung adenocarcinoma (ref. 1 and references therein). In subsequent studies, molecular mechanisms for these effects were analyzed, and it was found that cannabinoids inhibited tumor cell growth and induced apoptosis by modulating different cell signaling pathways in gliomas and lymphomas, prostate, breast, lung, skin, and pancreatic cancer cells (2–7). Encouraging data about the inhibition of tumor growth by cannabinoid receptor agonists in some animal tumor models, such as wistar rats inoculated with C6 gliomas (ref. 1 and references therein) and xenografts in athymic nude mice implanted with KiMol or MBA-MD-231 breast cancer cells, are beginning to emerge (5). The diversified effects of cannabinoids in modulating cell signaling pathways are depicted in Fig. 1. One puzzling caveat is that in contrast to the tumor-killing properties, some cannabinoids at low doses have also been shown to stimulate the growth of cancer cells *in vitro* (ref. 8 and references therein). Although the observed effects of cannabinoids are complex and sometimes contradictory, there is overwhelming evidence to suggest that cannabinoids can be explored as chemotherapeutic agents for the treatment of cancer (2–7).

Cannabinoids and Gliomas

Glioblastoma multiforme is one of the most dreadful forms of cancer and the most frequent class of malignant primary brain tumors. Antitumor action of two cannabinoid receptor agonists, $\Delta(9)$ -tetrahydrocannabinol and WIN-55,212-2 (a mixed CB₁/CB₂ agonist), was shown to be mediated through accumulation of ceramide, which resulted in sustained activation of extracellular signal-regulated kinase (ERK1/2; ref. 2 and references therein). It was also reported that cannabinoids down-regulated phosphoinositide 3-kinase (PI3K), protein kinase B (Akt), and ERK signaling pathways, and activated proapoptotic function of Bad protein, leading to the induction of apoptosis (9). It has also been shown that selective activation of the CB₂ receptor by JWH-133 mediates apoptosis in glioma cells via enhanced ceramide synthesis *de novo* (ref. 4 and references therein). In addition, a role for stress-regulated protein p8 (also designated as candidate of metastasis-1) and its downstream targets such as activating transcription factor-4 (ATF-4), CAAT/enhancer binding protein homologous protein,



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Figure 1. Schematic representation of signaling pathways associated with cannabinoid receptor activation induced by its agonists. Upon receptor binding, cannabinoid receptor agonists inhibit cell proliferation through inhibition of cAMP-dependent protein kinase (PKA), which activates mitogen-activated protein kinases (MAPK). Stimulation of ceramide synthesis via activation of serine palmitoyltransferase (SPT) up-regulates p8, leading to the subsequent induction of apoptosis. Cannabinoid receptor agonists also activates MAPKs and PI3K/Akt pathways; sustained activation of ERK1/2 leads to the induction of cyclin kinase inhibitor p27/KIP1 with modulation of cell cycle regulatory molecules, resulting in G₁ arrest and apoptosis. The proposed mechanisms are based on the available literature and are cell specific, and not all pathways are triggered simultaneously. Further studies are needed to unravel the detailed mechanism of action of cannabinoid receptor activation by their agonists. *CHOP*, CAAT/enhancer binding protein homologous protein; *PARP*, poly(ADP)ribose polymerase; *cdk*, cyclin-dependent kinase. *PKA*, cAMP-dependent kinase.

and cell death-inducible kinase (TRB3) was shown as a mechanism of the antitumor action of cannabinoids (2). A phase I clinical trial in nine patients with recurrent glioblastoma multiforme reported a fair safety profile of $\Delta(9)$ -tetrahydrocannabinol together with antiproliferative action on tumor cells (10). Contrary to these findings, Massi et al. (11) showed that cannabidiol treatment induces apoptosis in glioma cells *in vitro* and tumor regression *in vivo* through activation of caspases and reactive oxygen species via receptor-independent manner. Although there are few contradictory studies on the mechanism of action of cannabinoids, they all underline the importance of cannabinoids for the treatment of cancer. Hence, further studies are needed to elucidate the mechanism of action of cannabinoids in cancer treatment.

Cannabinoids and Prostate Cancer

The presence of cannabinoid receptors was shown in the prostate tissue and in prostate cancer PC-3 cells. However, it was shown that treatment of PC-3 cells with $\Delta(9)$ -tetrahydrocannabinol induced apoptosis via a receptor-independent manner (12). Interestingly, another study from the same group reported that activation of cannabinoid receptors in PC-3 cells stimulated the PI3K/Akt pathway with sequential involvement of Raf-1/ERK1/2 and nerve growth factor induction (ref. 13 and references therein). We have recently shown that the expression levels of both cannabinoid receptors CB₁ and CB₂ are significantly higher in human prostate cancer cells compared with normal prostate epithelial cells. Based on this observation, LNCaP cells were treated with WIN-55,212-2, which resulted in inhibition of cell growth and induction of apoptosis (ref. 4 and references therein) with an arrest of the cells in the G₀-G₁ phase of the cell cycle. This WIN-55,212-2-induced cell cycle arrest was associated with a sustained activation of ERK1/2 (4). To establish *in vivo* relevance of our *in vitro* findings, we showed that in CWR22Rv1 xenograft model, WIN-55,212-2-treated mice exhibited significant inhibition in the tumor growth with remarkable reduction of prostate-specific antigen secretion in the serum (14). Nithipatikom et al. (13) showed that increasing endogenous 2-arachidonoylgly and its stable analogue noladin ether inhibited invasion of androgen-independent prostate cancer PC-3 and DU-145 cells. Antiproliferative and apoptotic effects of endogenous cannabinoids anandamide in human prostate cancer cell lines LNCaP, DU145, and PC3 were found to be mediated through down-regulation of epidermal growth factor receptor (EGFR) and accumulation of ceramide (15). Interestingly, anandamide analogue (*R*)-methanandamide was shown to have a mitogenic effect on LNCaP cells at very low doses (16).

Cannabinoids and Breast Cancer

It has been shown that anandamide, potently and selectively, inhibited proliferation of human breast cancer cells. This antiproliferative activity of anandamide was accompanied by a reduction of cells in the S phase of the cell cycle and suppression of prolactin receptor (ref. 5 and references therein). Ligresti, Moriello, and colleagues (5) have shown antitumor activities of five natural cannabinoids, cannabidiol, cannabigerol, cannabichromene, cannabidiol acid, and $\Delta(9)$ -tetrahydrocannabinol, and suggested that cannabidiol was the most potent inhibitor of breast cancer cell growth. Both cannabidiol and the cannabidiol-rich extract also inhibited the growth of MDA-MB-231 breast carcinoma cells in athymic nude mice. In another study, (*R*)-methanandamide reduced the number and size of metastatic nodes, and this effect

was reversed by CB₁ receptor antagonist SR141716A. (*R*)-methanandamide-treated cells also showed decreased phosphorylation of focal adhesion-associated protein kinase and Src, and tyrosine kinases involved in migration and adhesion, suggesting that CB₁ receptor activation might represent a novel therapeutic strategy to slow down the growth of breast carcinoma and to inhibit its metastatic diffusion *in vivo* (17). Contrary to these findings, McKallip et al. (18) have earlier shown that $\Delta(9)$ -tetrahydrocannabinol enhanced breast cancer growth and metastasis specifically in cells expressing low levels of cannabinoid receptors by suppressing the antitumor immune response, suggesting that cannabinoid exposure may increase the incidence of breast cancer as well as other cancers that do not express cannabinoid receptors.

Cannabinoid and Lung Cancer

Lung cancer survival figures argue powerfully for new approaches to control this disease by agents that could reverse, suppress, or completely halt tumor development. Guzman (ref. 1 and references therein) reported for the first time that Lewis lung adenocarcinoma growth was retarded by the p.o. administration of $\Delta(9)$ -tetrahydrocannabinol, and based *in vitro* studies, inhibition of DNA synthesis was identified as a mechanism for these effects. Another study showed that concentrations of $\Delta(9)$ -tetrahydrocannabinol comparable with those detected in the serum of patients after $\Delta(9)$ -tetrahydrocannabinol administration accelerate proliferation of lung cancer cells (ref. 8 and references therein). Treatment of lung carcinoma cell line NCI-H292 with nanomolar concentrations of $\Delta(9)$ -tetrahydrocannabinol led to accelerated cell proliferation that was dependent on EGFR-mediated activation of ERK1/2 as well as PKB/Akt signaling (ref. 8 and references therein). Recently, it has been shown that $\Delta(9)$ -tetrahydrocannabinol treatment inhibited epidermal growth factor-induced phosphorylation of ERK1/2, c-Jun-NH₂-kinase1/2, and Akt in A549 human lung cancer cell line as well as suppression of metastasis and s.c. tumor growth in severe combined immunodeficient mice (8).

Cannabinoid and Skin Cancer

Melanoma is responsible for the greatest number of skin cancer-related deaths worldwide. It was reported that CB₁ and the CB₂ receptors are expressed in normal skin and skin tumors of mice and humans. *In vitro* studies showed that activation of cannabinoid receptors induced the apoptotic death of tumorigenic epidermal cells, without affecting the nontransformed epidermal cells. Administration of WIN-55,212-2 or the selective CB₂ agonist JWH-133 was shown to result in growth inhibition of malignant tumors in nude mice (ref. 6 and references therein). Another study showed that activation of these receptors decreased tumor growth, angiogenesis and metastasis of melanomas in mice, and inhibited proliferation via inhibition of Akt pathway and hypophosphorylation of retinoblastoma in melanoma cells (6). These two studies offer an exciting opportunity to further explore the use of cannabinoids for the treatment and management of melanoma.

Cannabinoid and Pancreatic Cancer

Pancreatic cancer ranks as one of the most fatal forms of cancer, and therefore, new strategies aimed at improving the prognosis of this deadly disease are warranted. Recently, it was shown that cannabinoid administration leads to apoptosis of pancreatic tumor cells via CB₂ receptor and ceramide-dependent up-regulation of p8

and ATF-4 and TRB3 stress-related genes (7). Another study showed that CB₁ receptor antagonist AM251-induced cell death in pancreatic MIA PaCa-2 cells occurred via receptor-independent manner (19). Although the two studies describe contrasting mechanism of action of cannabinoids, both underline the importance of cannabinoids for the treatment of pancreatic cancer. In depth studies are therefore warranted to identify the mechanism of action of cell death induced by cannabinoids in pancreatic cancer.

Cannabinoid and Lymphoma

Studies show that exposure of murine lymphoma tumors EL-4, LSA, and P815 to $\Delta(9)$ -tetrahydrocannabinol *in vitro* led to a significant reduction in cell viability and an increase in apoptosis, and EL-4 tumor-bearing mice led to a significant reduction in tumor load, increase in tumor-cell apoptosis, and increase in survival of tumor-bearing mice (ref. 20 and references therein). Similar observations were made by Flygare et al. (20) who treated mantle cell lymphoma (MCL) cells with cannabinoid receptor ligands and found a decrease in cell viability, whereas control cells lacking CB₁ were not affected. Recently, Gustafsson et al. (3) reported that cannabinoid receptor-mediated apoptosis induced by (*R*)-methanandamide and WIN-55,212-2 in MCL was associated with ceramide accumulation and p38. These data suggest that targeting CB₁ and CB₂ receptors by their agonists may have therapeutic potential for the treatment of lymphoma.

Conclusions and Future Prospects: Promise

Cannabinoids, the active components of marijuana and their other natural and synthetic analogues have been reported as useful adjuvants to conventional chemotherapeutic regimens for preventing nausea, vomiting, pain, and for stimulating appetite.

Before the discovery of specific cannabinoid systems and receptors, it was speculated that cannabinoids produced their effects via nonspecific interaction with cell membranes. Cannabinoids are proving to be unique based on their targeted action on cancer cells and their ability to spare normal cells. Variation in the effects of cannabinoids in different cell lines and tumor model could be due to the differential expression of CB₁ and CB₂ receptors. Thus, overexpression of cannabinoid receptors may be effective in killing tumors, whereas low or no expression of these receptors could lead to cell proliferation and metastasis because of the suppression of the antitumor immune response. It is also reported that low doses of cannabinoid administration accelerate proliferation of cancer cells instead of inducing apoptosis and, thereby, contribute to cancer progression. Till date, very little is known about the mechanism of action of cannabinoids. There is need for further in-depth studies to elucidate the precise mechanism of cannabinoid action in cancer cells. Safety of $\Delta(9)$ -tetrahydrocannabinol administration has been determined, and a dose escalation regimen showed that cannabinoid delivery was safe and could be achieved without overt psychoactive effects. In view of the fair safety profile of most cannabinoids together with their antiproliferative action on tumor cells, clinical trials are required to determine whether cannabinoids could be used for the inhibition of tumor growth in a clinical setting. If this could be established, then one can hope that nontoxic, nonhabit forming cannabinoids could be developed as novel therapeutic agents for the treatment of cancer.

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